

Morphological and physiological differences in heteromorphic leaves of male and female *Populus euphratica* Oliv.

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Abstract: Leaf traits can directly reflect the adaptation strategies of plants to the environment. However, there is limited knowledge on the adaptation strategies of heteromorphic leaves of male and female *Populus euphratica* Oliv. in response to individual developmental stages (i.e., diameter class) and canopy height changes. In this study, morphological and physiological properties of heteromorphic leaves of male and female *P. euphratica* were investigated. Results showed that both male and female *P. euphratica* exhibited increased leaf area (LA), leaf dry weight (LDW), leaf thickness (LT), net photosynthetic rate (P_n), transpiration rate (T_r), stomatal conductance (g_s), proline (Pro), and malondialdehyde (MDA) concentration, decreased leaf shape index (LI) and specific leaf area (SLA) with increasing diameter and canopy height. Leaf water potential (LWP) increased with increasing diameter, LWP decreased significantly with increasing canopy height in both sexes, and carbon isotope fraction ($\delta^{13}C$) increased significantly with canopy height in both sexes, all of which showed obvious resistance characteristics. However, males showed greater LA, LT, P_n , T_r , and Pro than females at the same canopy height, and males showed significantly higher LA, SLA, LT, P_n , T_r , g_s , and MDA, but lower LWP and $\delta^{13}C$ than females at the same canopy height, suggesting that male *P. euphratica* have stronger photosynthetic and osmoregulatory abilities, and are sensitive to water deficiency. Moreover, difference between male and female *P. euphratica* is closely related to the increase in individual diameter class and canopy height. In summary, male plants showed higher stress tolerance than female plants, and differences in P_n , g_s , T_r , Pro, MDA, $\delta^{13}C$, and LWP between females and males were related to changes in leaf morphology, diameter class, and canopy height. The results of this study provide a theory for the differences in growth adaptation strategies during individual development of *P. euphratica*.

Keywords: arid area; canopy height; dioecious plants; morphology; physiological characteristics

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1 Introduction

Leaf traits are highly ecologically plastic and can effectively reflect plant adaptation under different environmental conditions (Funk et al., 2013). Plants that exhibit different morphology of leaves at different growth periods or under different environmental conditions are known as heteromorphic leaves (Bai, 2003). Heteromorphic leaves play a crucial role in the adaptation to

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environmental changes, for instance, changes in leaf morphology in aquatic plants facilitate gas exchange under submerged conditions (Kuwabara and Nagata, 2002; Mommer and Visser, 2005). Previous studies have shown that changes in leaf morphological characteristics are related to tree height, as light utilization and evapotranspiration requirements change significantly with tree height (Russo and Kitajima, 2016). Kenzo et al. (2015) found a significant linear relationship among leaf morphology, biochemical properties, and tree height. In addition, Zhai et al. (2020) found that morphological and physiological characteristics of poplar leaves exhibit significant differences as individual developmental stages and tree height increase, as an adaptation to water stress. In higher plants, water stress causes the accumulation of osmoregulatory substances such as proline (Pro) and malondialdehyde (MDA) (Kishor et al., 2005). Koch et al. (2004) found that leaf water potential (LWP) decreases with increasing tree height, and that this decrease reduces leaf expansion and photosynthesis. The latter change was directly caused by increasing $\delta^{13}\text{C}$ and indirectly by changing leaf structure under water stress condition.

P. euphratica belongs to the genus *Populus* in the Salicaceae family, and is an important dioecious tree with heteromorphic leaf (Wei, 1999), which is widely distributed in arid and semi-arid desert areas. *P. euphratica* plays a key role in ecosystem function, sand fixation, and climate regulation in oases (Keyimu et al., 2018). In order to improve the adaptability to the arid environment, *P. euphratica* changes the morphological and functional traits of the leaves, such as gradually increasing leaf area and leaf thickness, decreasing specific leaf area, and increasing dry matter content and leaf dry weight (Huang et al., 2010; Wang et al., 2011). Previous studies found that heteromorphic leaves of *P. euphratica* had enhanced photosynthetic capacity, higher content of osmoregulatory substances, and stronger osmoregulatory capacity with the increase of tree height under water stress (Zhai et al., 2020). The morphological, physiological, and biochemical characteristics of heteromorphic leaves of *P. euphratica* have been extensively studied (Liu et al., 2015; Li et al., 2017; Zhai et al., 2020). And most studies did not take into account sex differences of plants. However, previous studies have found that plants exhibited sex differences in morphological characteristics, resource use efficiency, and other aspects (Espíto-Santo et al., 2003; Lei et al., 2017), and differences in morphological and physiological characteristics between male and female plants in different environments are important factors influencing the composition, structure, and distribution of plant populations (Li et al., 2019). The adaptive characteristics and sex differences in heteromorphic leaves of female and male *P. euphratica* have not been reported yet. Therefore, we investigated the differences in morphological characteristics, photosynthesis, water use efficiency, and osmoregulatory substances of heteromorphic leaves of male and female *P. euphratica* under different developmental stages and canopy heights in order to elucidate their adaptation strategies of heteromorphic leaves. We focus on the following questions: (1) what is the pattern of variation in the morphological and physiological characteristics of the heteromorphic leaves of female and male *P. euphratica* during individual developmental stages? (2) are there any sex differences in the morphological and physiological characteristics of the heteromorphic leaves of *P. euphratica* at different diameter classes and canopy heights? and (3) are the morphological and physiological differences of heteromorphic leaves between females and males related to individual diameter and canopy height? The results of this study might provide a theoretical basis for exploring the adaptation strategies of heteromorphic leaves of both male and female *P. euphratica* during individual developmental stage.

2 Materials and methods

2.1 Study area

The study area is located in an artificial *P. euphratica* forest (40°32'36"N, 81°17'56"E; 980 m a.s.l.) at the northwestern edge of the Tarim Basin, Xinjiang, with male and female *P. euphratica* covering different diameter classes. In late March 2020, when *P. euphratica* began to flower, we

identified and tagged male and female individuals according to their different inflorescences (Fig. S1). The climate of the study area is hot and dry, with an average annual precipitation of about 50 mm, an annual average temperature of 10.80°C, a potential evaporation of more than 1900 mm, and an average annual sunshine hours of 2900 h. It is a typical temperate continental hyperarid desert climate zone. The habitat conditions include 27.53% soil water content, 1.50 m groundwater depth, 27.53% soil humidity, 31.09°C daily average air temperature, and 36.78% daily average air humidity.

2.2 Experimental design and sampling

To reduce the influence of environmental factors, we selected male and female *P. euphratica* of different diameter classes under the same habitat condition. The sample plants were divided into different diameter classes by using diameter at breast height (DHB) at intervals of 4 cm. Three females and three males of 8, 12, 16, and 20 cm diameter classes were selected from the study site, and DBH and height of the sample plants were measured using a tape measure and a laser height measuring device (Haglöf, Sweden; Table S1). DBH and mean age (A) for each diameter class of female and male *P. euphratica* fit the relationship: $A = 13.679 / (1 + 3.3476 \times \exp(-0.2099 \text{DBH}))$ (Gu et al., 2013). Starting from the base of the main trunk of each plant (near the ground), we set sampling points at 2 m intervals along the base of the canopy toward the top (2, 4, 6, 8, 10, and 12 m, i.e., the canopy height of sampling points). Considering the influence of light and other factors on the growth of leaves in different directions, we collected three annual branches at each sampling point from the eastern, southern, western, and northern directions, and selected the leaves at the 4th node of each branch from the base to the top as sample leaves for the determination of morphological and physiological parameters of heteromorphic leaves.

2.3 Measurement of leaf morphological parameters

Leaf length (LL), leaf width (LW), and leaf area (LA) were measured using the portable leaf area meter LI-3000C (Li-Cor Inc., Lincoln, USA). Leaf thickness (LT) is measured using a vernier caliper (3 measurements at the top, middle, and bottom of the leaf, and averaged to obtain the leaf thickness). Sample leaves were heated at 105 °C for 10 min, then at 80 °C for a constant weight, and leaves were weighed to obtain the leaf dry weight (LDW). Leaf shape index (LI) was calculated from leaf length/width ratio (Tsukaya, 2002), and specific leaf area (SLA) (leaf area/leaf dry weight) was calculated (Roderick et al., 1999).

2.3 Measurement of leaf stomatal exchange parameters

Using the LI-6400 photosynthesis measurement system (Li-Cor Inc., Lincoln, USA), we measured the net photosynthetic rate (P_n), stomatal conductance (g_s), intercellular CO₂ concentration (C_i), and transpiration rate (T_r) of leaves at 09:30–11:30 (LST). The leaves were measured with a light quantum flux density of 1200 $\mu\text{mol}/(\text{m}^2 \cdot \text{s})$, a leaf temperature of 25 °C, and an ambient CO₂ concentration of 400 $\mu\text{mol}/\text{mol}$, and the instantaneous water use efficiency (WUE) was calculated from the net photosynthetic rate/transpiration rate, namely, $\text{WUE} = P_n/T_r$ (Zhai et al., 2020). Twelve leaves were measured at each sampling site, and the measurements were repeated three times.

2.5 LWP and carbon isotopic composition ($\delta^{13}\text{C}$) measurements

Leaf was selected from the 4th node of each branch from the base towards the tip, and LWP was measured using a portable plant water pressure chamber (600-EXP). Measurements were made before dawn and took no more than 2 h. Sample leaves on selected branches are cut and measured immediately thereafter, with 12 leaves measured at each sampling point and averaged. After immediately measuring the morphological parameters of the leaves of each sample tree, we rinsed the samples with distilled water, subjected to 105°C for 10 min and dried in an oven at 60°C for 48 h to a constant weight. A pulveriser was used to pulverise and pass the dried product through a 100-mesh sieve, and a glass vacuum system was used to prepare the plant samples for carbon isotope analysis. The combustion furnace was connected to the power supply, and the furnace

temperature was maintained at 1000°C. After evacuation, oxygen was supplied to the system. A porcelain spoon containing the sample was placed in a combustion tube and burned at high temperature for 2 min. The CO₂ gas was then collected and purified by freezing and analyzed for carbon isotope composition using an isotope mass spectrometer (Finnigan MAT, San Jose, CA, USA).

2.6 Determination of leaf Pro and MDA concentrations

A mixture of leaves from the 4th node of annual branches in the same canopy was selected as the test sample and measured in microvolume using a kit (Suzhou Kemin Biotechnology Co., Ltd., China) for the determination of leaf Pro and MDA concentrations.

2.7 Data analysis

Tests of normality were carried out prior to statistical comparisons, and data were log-transformed to correct deviations. SPSS v.23.0 (SPSS, Chicago, IL, USA) was used to test for individual differences between means at a significance level of $P < 0.05$. A multi-factor ANOVA was also used to test for the effects of sex, canopy height, diameter classes, and their interactions. And principal component analysis (PCA) was conducted using Canoco 5 (Microcomputer Power, USA) to further reveal the effects of individual developmental stage and canopy height on leaf traits between sexes.

3 Results

3.1 Sex differences in morphological characteristics of heteromorphic leaves

LA and LDW of male and female *P. euphratica* increased significantly with increasing diameter classes, and SLA decreased significantly. In addition, LA and LT were significantly influenced by sex (Table 1). LA and LT differ significantly between males and females at the same diameter class, with both LA and LT being higher in males than in females, but LI, SLA, and LDW are not significantly different between males and females. As canopy height increased, LI, LA, and LT increased significantly in both sexes, and SLA decreased significantly in both sexes. In addition, sex, canopy height, and their interactions had significant effects on LA, SLA, and LT (Fig. 1). LA, SLA, and LT of heteromorphic leaves were all significantly greater in males than in females at the same canopy height. These results indicate that with increasing individual developmental stage and canopy height, male plants exhibit larger and thicker leaves than female plants.

Table 1 Comparison of morphological characteristics of heteromorphic leaves of male and female *P. euphratica* at different developmental stages

Sex	DC	LI	LA (cm ²)	SLA (cm ² /g)	LT (mm)	LDW (g)
Female	8	2.45 ± 0.77 ^a	10.52 ± 2.03 ^{de}	107.31 ± 6.37 ^a	0.35 ± 0.01 ^{bc}	0.14 ± 0.04 ^c
	12	2.63 ± 0.67 ^a	13.99 ± 2.96 ^d	96.79 ± 10.00 ^{ab}	0.35 ± 0.02 ^{bc}	0.15 ± 0.03 ^{bc}
	16	2.37 ± 0.89 ^{ab}	12.22 ± 4.36 ^e	96.91 ± 15.51 ^{ab}	0.38 ± 0.03 ^b	0.15 ± 0.04 ^{bc}
	20	1.84 ± 0.19 ^{abc}	22.41 ± 2.32 ^{bc}	83.83 ± 9.77 ^b	0.38 ± 0.03 ^b	0.25 ± 0.03 ^a
Male	8	1.73 ± 0.68 ^{bc}	14.58 ± 2.71 ^e	113.31 ± 11.69 ^a	0.38 ± 0.02 ^b	0.14 ± 0.06 ^c
	12	2.02 ± 0.34 ^a	15.28 ± 2.75 ^{cde}	108.09 ± 9.34 ^a	0.39 ± 0.02 ^{ab}	0.18 ± 0.03 ^b
	16	1.85 ± 0.31 ^{abc}	18.75 ± 5.07 ^{cd}	106.31 ± 16.13 ^a	0.41 ± 0.04 ^a	0.18 ± 0.05 ^b
	20	1.68 ± 0.17 ^c	30.04 ± 2.57 ^a	89.61 ± 8.96 ^b	0.41 ± 0.04 ^a	0.27 ± 0.05 ^a
P_S		*	**	ns	**	ns
P_{DC}		ns	***	*	ns	**
$P_S \times P_{DC}$		ns	ns	ns	ns	ns

Note: DC, diameter class; LI, leaf index; LA, leaf area; SLA, specific leaf area; LT, leaf thickness; LDW, leaf dry weight; P_S , sex effect; P_{DC} , diameter class effect; $P_S \times P_{DC}$, sex and diameter class interactive effect. Different lowercase letters within the same column indicate significant difference among females or males at $P < 0.05$ level. Mean ± SE, $n = 108$. ns, not significant; *, $P < 0.05$ level; **, $P < 0.01$ level; ***, $P < 0.001$ level.

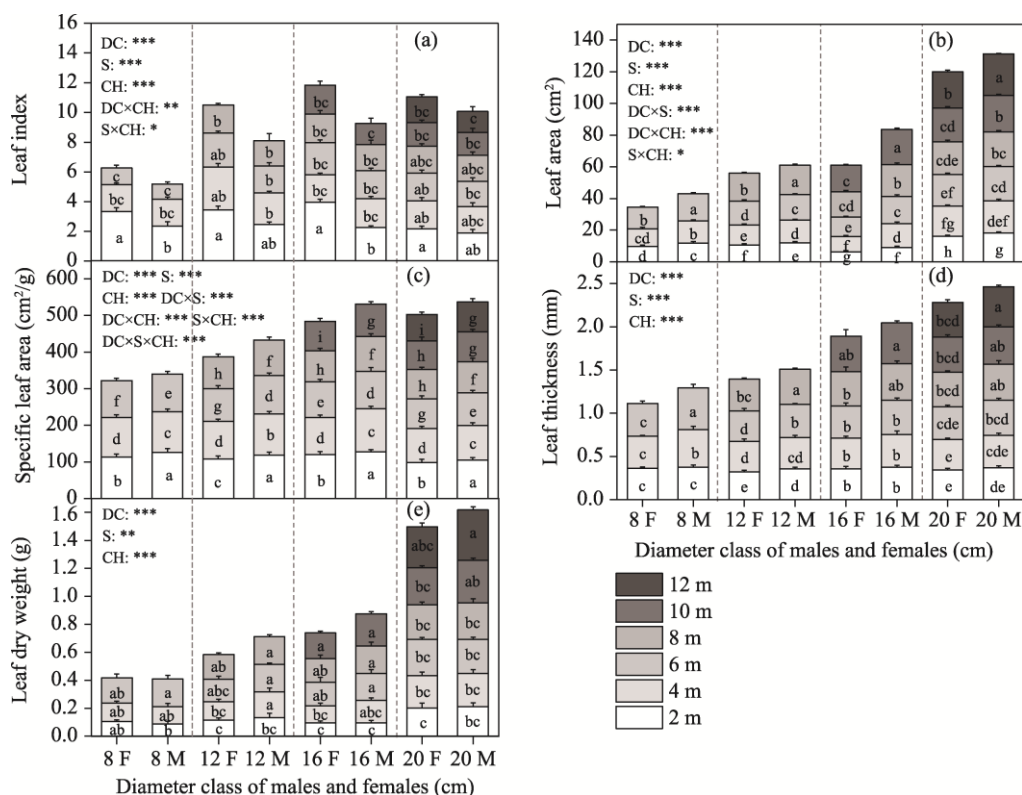


Fig. 1 Comparison of morphological characteristics of heteromorphic leaves between male and female *P. euphratica* at different canopy heights and diameter classes. Different lowercase letters within the same class are significant differences among different heights at $P<0.05$ level. 8 F–20 F, females of 8–20 cm diameter classes; 8 M–20 M, males of 8–20 cm diameter classes. S, sex; CH, canopy height; DC, diameter class; S×DC, interaction of sex and diameter class; DC×CH, interaction of diameter class and canopy height; S×CH, interaction of sex and canopy height; S×DC×CH, interaction among sex, diameter class, and canopy height. *, $P<0.05$ level; **, $P<0.01$ level; ***, $P<0.001$ level. The abbreviations are the same as in the following figures.

3.2 Sex differences in photosynthetic characteristics of heteromorphic leaves

P_n , T_r , C_i , and T_r of heteromorphic leaves of different diameter classes were influenced by sex (Fig. 2), with significant differences in P_n and T_r between male and female *P. euphratica* in the 12 cm diameter classes. As canopy height increased, P_n , g_s , and T_r increased significantly in both sexes (Fig. 3a–c). C_i and WUE decreased significantly (Fig. 3d and e). In addition, the interaction among sex, canopy height, and diameter classes had significant effects on P_n , T_r , and g_s (Fig. 3). The differences in P_n , g_s , and T_r between male and female *P. euphratica* at the same canopy height were significant, and P_n , g_s , and T_r were significantly greater in males than in females, indicating that male *P. euphratica* exhibit higher photosynthetic capacity than female plants.

3.3 Sex differences in water physiological characteristics of heteromorphic leaves

Figure 4 showed the water characteristics of heteromorphic leaves of male and female *P. euphratica*. $\delta^{13}C$ of heteromorphic leaves at different diameter classes was significantly influenced by sex (Fig. 4c). In the 8–16 cm diameter classes, the difference in $\delta^{13}C$ of heteromorphic leaves between male and female *P. euphratica* was significant, and $\delta^{13}C$ of female plants was higher than that of male plants, indicating that long-term WUE of female plants was higher than that of male plants at the same developmental stage. In addition, LWP of both sexes was significantly influenced by diameter class, with LWP of both sexes at 20 cm diameter class being significantly higher than the rest of diameter classes (Fig. 4a), indicating that LWP of both sexes tended to increase as the individual developmental stage increased. As canopy height increased, LWP decreased significantly, and $\delta^{13}C$ increased significantly in both male and female

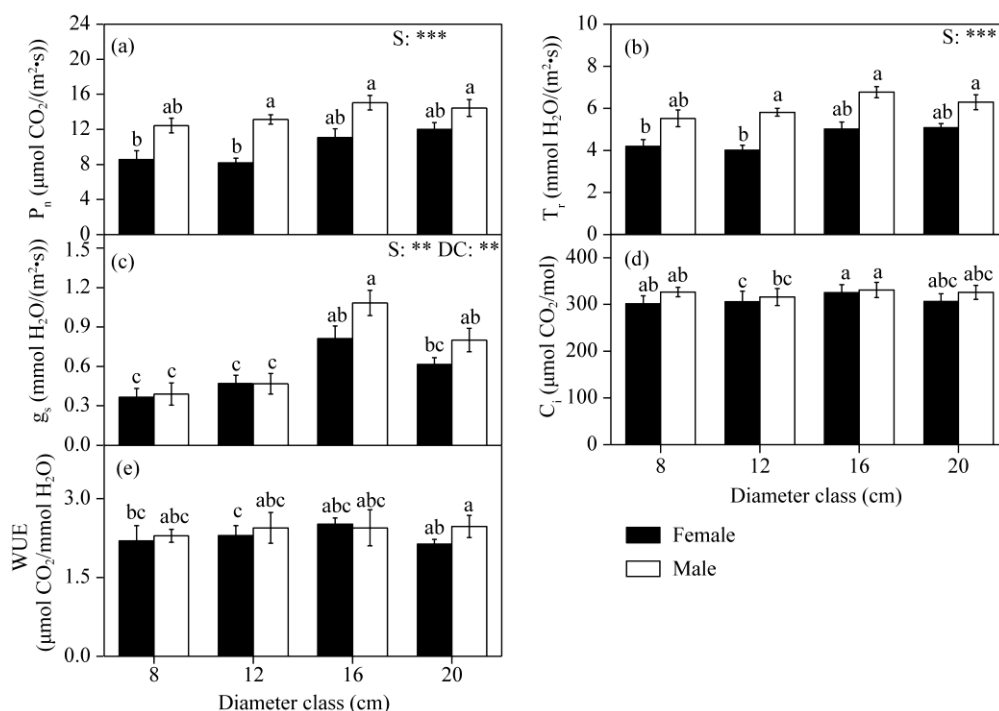


Fig. 2 Comparison of photosynthetic characteristics of heteromorphic leaves between male and female *P. euphratica* at different developmental stages. P_n , net photosynthetic rate (a); T_r , transpiration rate (b); g_s , stomatal conductance (c); C_i , intercellular CO_2 concentration (d); WUE, water use efficiency (e). Different lowercase letters indicate significant differences among different diameter classes at $P < 0.05$ level. The abbreviations are the same as in the following figures.

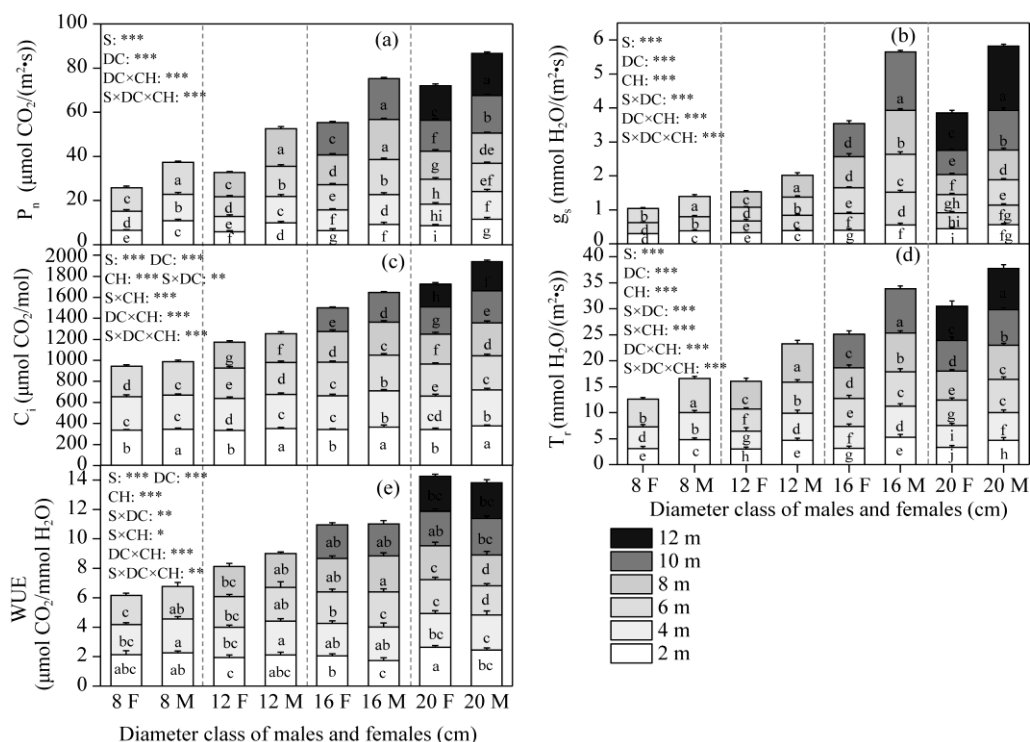


Fig. 3 Comparison of photosynthetic characteristics of heteromorphic leaves between male and female *P. euphratica* at different canopy heights and diameter classes. P_n , net photosynthetic rate (a); g_s , stomatal conductance (b); C_i , intercellular CO_2 concentration (c); T_r , transpiration rate (d); WUE, water use efficiency (e).

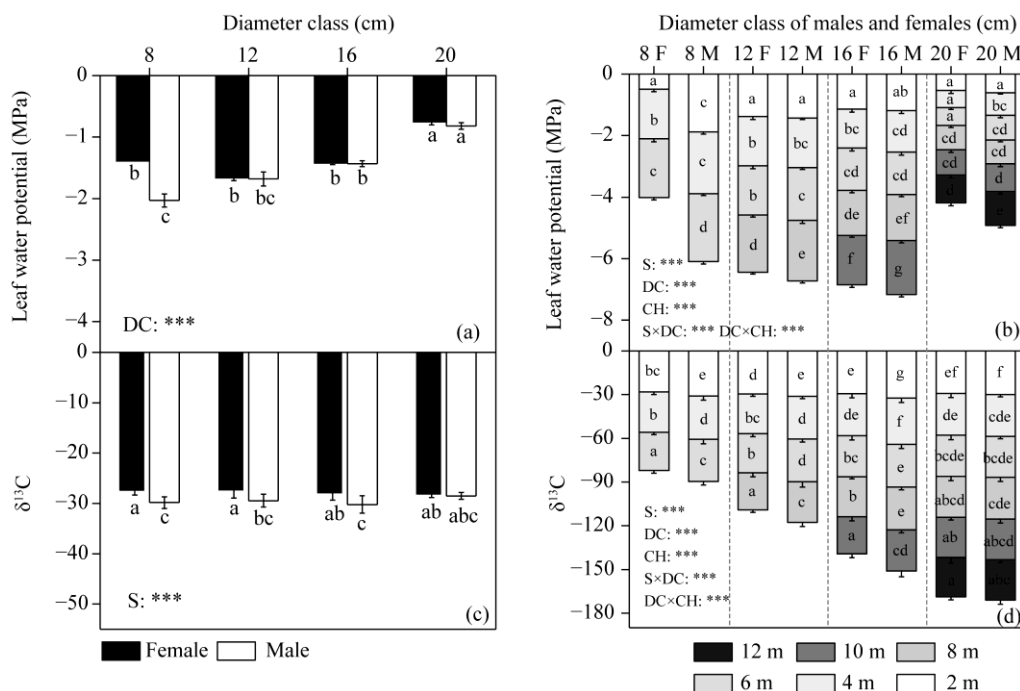


Fig. 4 Comparison of water physiological characteristics of heteromorphic leaves between male and female *P. euphratica* at different canopy heights and diameter classes. (a and b), leaf water potential; (c and d), $\delta^{13}\text{C}$.

plants. In addition, sex, diameter classes, canopy height, and their interactions had significant effects on LWP and $\delta^{13}\text{C}$ (Fig. 4b and d). Differences in LWP and $\delta^{13}\text{C}$ between male and female plants at the same canopy height were significant, with both LWP and $\delta^{13}\text{C}$ higher in females than in males, suggesting that males had a greater capacity for water uptake, while females had more water efficiency in a long-term period.

3.4 Sex difference in Pro and MDA of heteromorphic leaves

Pro and MDA concentrations in heteromorphic leaves of male and female *P. euphratica* tended to increase with increasing diameter classes (Fig. 5), and differences in heteromorphic leaf between males and females at different diameter classes were not significant (Fig. 5a and c). As canopy height increased, the concentrations of Pro and MDA increased in both sexes, with significant differences in Pro and MDA concentrations at the top and bottom of the canopy, with those at the top being significantly higher than those at the bottom. In addition, at different canopy heights, the concentrations of MDA and Pro were significantly influenced by sex (Fig. 5b and d), with significant differences in Pro concentrations between males and females at the same canopy height in the 12 diameter classes, and higher concentrations of Pro in males than in females. In addition, sex, diameter class, canopy height, and their interaction had significant effects on MDA (Fig. 5d). Difference in MDA concentration between male and female *P. euphratica* at the same canopy height was significant, and all male *P. euphratica* had higher MDA concentrations than female plants, indicating that male plants were more sensitive to stress.

3.5 Relationship among functional traits of heteromorphic leaves, developmental stage, and canopy height

Correlation among functional traits of heteromorphic leaves of male and female *P. euphratica*, developmental stage, and canopy height is shown in Table 2. LA, LDW, and g_s of heteromorphic leaves of both sexes were positively correlated with DC and CH. SLA was negatively correlated with DC and CH. LT, P_n , T_r , Pro, MDA, and $\delta^{13}\text{C}$ were positively correlated with CH, while LI and C_i were negatively correlated with CH in heteromorphic leaves of both sexes. LWP of female

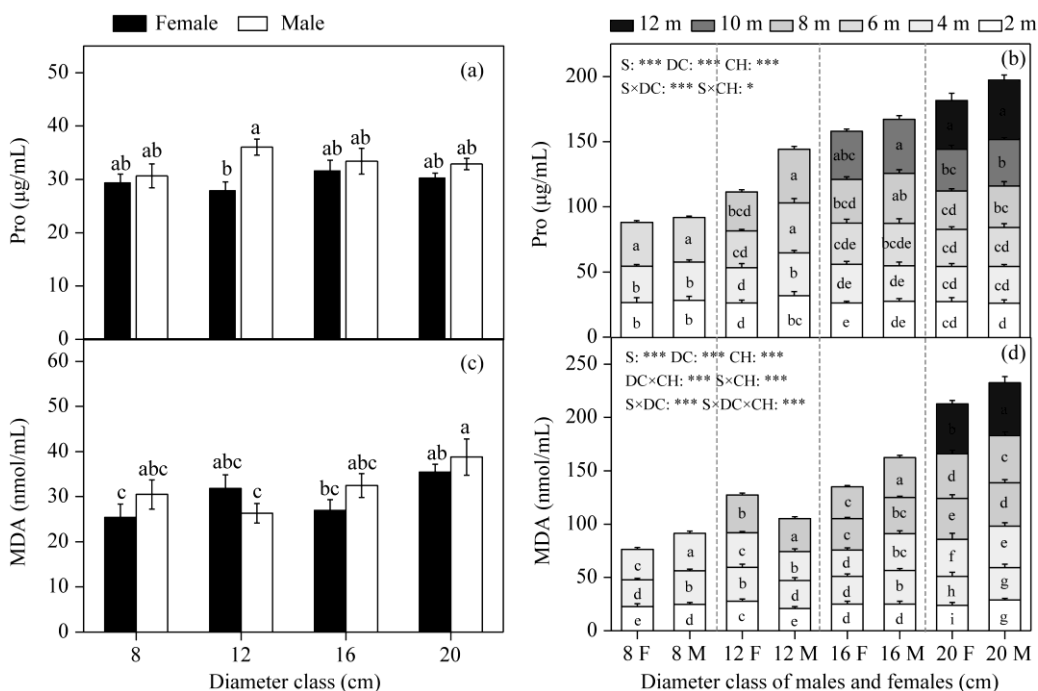


Fig. 5 Comparison of proline (Pro, a and b) and malondialdehyde (MDA, c and d) concentrations in heteromorphous leaves between male and female *P. euphratica* at different canopy heights and diameter classes

Table 2 Correlation among functional trait of heteromorphous leaves between male and female *P. euphratica*, developmental stage, and canopy height

Sex	Index	LI	LA	LDW	SLA	LT	P _n	g _s	C _i	T _r	WUE	Pro	MDA	δ ¹³ C	LWP
Female	DC	-0.23	0.63**	0.71**	-0.60**	0.31	0.52*	0.54*	-0.20	0.36	0.78**	0.24	0.43	-0.29	-0.86**
Female	CH	-0.63**	0.75**	0.70**	-0.89**	0.87**	0.94**	0.83**	-0.94**	0.96**	0.28	0.86**	0.81**	0.68**	0.00
Male	DC	-0.17	0.65**	0.68**	-0.61**	-0.01	0.27	0.51*	0.00	0.28	0.24	0.03	0.55*	0.32	0.91**
Male	CH	-0.72**	0.85**	0.81**	-0.86**	0.74**	0.92**	0.83**	-0.88**	0.92**	0.34	0.85**	0.86**	0.80**	0.02

Note: DC, diameter class; CH, canopy height; LI, leaf index; LA, leaf area; SLA, specific leaf area; LT, leaf thickness; LDW, leaf dry weight; P_n, net photosynthetic rate; g_s, stomatal conductance; C_i, intercellular CO₂ concentration; T_r, transpiration rate; WUE, water use efficiency; Pro, proline; MDA, malondialdehyde content; LWP, leaf water potential. * $P < 0.05$ level; ** $P < 0.01$ level. The abbreviations are the same as in Table 3.

plants was negatively correlated with DC, while LWP of male plants was positively correlated with DC. In addition, P_n and WUE of female plants were positively correlated with DC, while MDA of male plants was positively correlated with DC.

LI and SLA in heteromorphous leaves of both sexes were negatively correlated with P_n, T_r, Pro, and δ¹³C, positively correlated with C_i. LA, LDW, and LT all showed positive correlations with P_n, g_s, T_r, Pro, and MDA, and significantly negative correlations with C_i (Table 3). In addition, LA of female plants was positively correlated with WUE, and negatively correlated with LWP. WUE was positively correlated with LDW and negatively correlated with SLA. LI of male plant was negatively correlated with g_s and MDA.

3.6 PCA of functional traits of heteromorphous leaves

To reveal the main factors influencing the variation in functional traits of heteromorphous leaves between males and females, we carried out PCA using indicators of each functional trait of heteromorphous leaves. Results showed that female and male plants were well separated from each other at different developmental stages and canopy heights (Fig. 6). Two principal components of PCA explained 75.73% (Fig. 6a) and 98.95% (Fig. 6b) of the total variation, respectively. At different developmental stages, female and male plants are separated from each other along the

Table 3 Correlation between functional traits of heteromorphic leaves of male and female *P. euphratica*

Index	LI	LA	LDW	SLA	LT	P _n	g _s	C _i	T _r	WUE	Pro	MDA	δ ¹³ C	LWP
LI	1.00	-0.72**	-0.67**	0.72**	-0.87**	-0.70**	-0.52*	0.62**	-0.70**	-0.39	-0.53*	-0.74**	-0.76**	0.10
LA	-0.65**	1.00	0.94**	-0.97**	0.59**	0.78**	0.73**	-0.63**	0.73**	0.52*	0.57*	0.88**	0.87**	0.40
LDW	-0.66**	0.96**	1.00	-0.92**	0.50*	0.69**	0.68**	-0.58**	0.62**	0.44	0.57*	0.87**	0.82**	0.48*
LSA	0.68**	-0.93**	-0.87**	1.00	-0.60**	-0.76**	-0.66**	0.68**	-0.73**	-0.42	-0.58**	-0.85**	-0.92**	-0.37
LT	-0.59**	0.59**	0.60**	-0.71**	1.00	0.70**	0.62**	-0.67**	0.75**	0.30	0.61**	0.73**	0.61**	-0.29
P _n	-0.72**	0.78**	0.77**	-0.90**	0.90**	1.00	0.85**	-0.81**	0.93**	0.51*	0.83**	0.74**	0.72**	-0.10
g _s	-0.42	0.57*	0.58**	-0.74**	0.77**	0.89**	1.00	-0.57*	0.85**	0.42	0.67**	0.78**	0.47*	0.17
C _i	0.54*	-0.68**	-0.59**	0.81**	-0.75**	-0.82**	-0.70**	1.00	-0.82**	-0.13	-0.90**	-0.61**	-0.77**	0.32
T _r	-0.73**	0.69**	0.68**	-0.84**	0.90**	0.97**	0.85**	-0.85**	1.00	0.22	0.82**	0.74**	0.66**	-0.11
WUE	-0.24	0.62**	0.55*	-0.56*	0.09	0.33	0.31	-0.25	0.15	1.00	0.14	0.45	0.38	0.16
Pro	-0.55*	0.52*	0.52*	-0.67**	0.76**	0.86**	0.89**	-0.82**	0.87**	0.13	1.00	0.52*	0.65**	-0.30
MDA	-0.41	0.81**	0.77**	-0.81**	0.58**	0.67**	0.52*	-0.78**	0.65**	0.37	0.57*	1.00	0.72**	0.30
δ ¹³ C	-0.51*	0.36	0.22	-0.49*	0.57*	0.54*	0.36	-0.80**	0.65**	0.00	0.58**	0.41	1.00	0.10
LWP	-0.07	0.48*	0.60**	-0.30	0.04	0.18	0.17	0.15	-0.02	0.57*	-0.10	0.23	-0.59**	1.00

first principal component (PC1) analysis axis; at different canopy heights, female and male plants are separated from each other along the second principal component (PC2) analysis axis. PC1 was more influenced by LA, LT, LDW, SLA, δ¹³C, LWP, g_s, P_n, T_r, and MDA, while PC2 was more influenced by LI, C_i, WUE, and Pro. The results showed that LA, LT, P_n, T_r, g_s, MDA, and Pro of male plant showed more pronounced changes at different developmental stages and canopy heights than female plants.

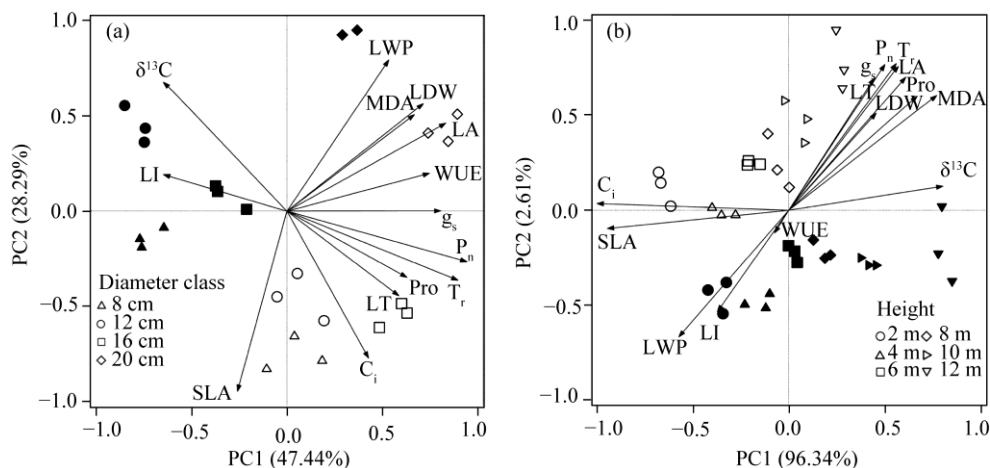


Fig. 6 Principal component analysis (PCA) of functional traits of heteromorphic leaves between male and female plants at different developmental stages (a) and canopy heights (b). Open symbols, male plants; filled symbols, female plants. PC, principal component; LI, leaf index; LA, leaf area; LT, leaf thickness; SLA, specific leaf area; LDW, leaf dry weight; P_n, net photosynthetic rate; g_s, stomatal conductance; C_i, intercellular CO₂ concentration; T_r, transpiration rate; WUE, water use efficiency; Pro, proline; MDA, malondialdehyde content; LWP, leaf water potential.

4 Discussion

In this study, significant differences were found in LA, SLA, LT, P_n, T_r, g_s, LWP, δ¹³C, Pro, and MDA of heteromorphic leaves of male and female *P. euphratica* as individual developmental stage and canopy height changed. This sex difference was closely related to the increases in individual developmental stage and canopy height, especially for the different canopy heights. At

the same canopy height, male plants showed higher LA, SLA, LT, P_n , T_r , g_s , Pro, and MDA, and lower LWP and $\delta^{13}C$ compared with female plants, indicating that at different canopy heights, male plants showed more morphological and physiological adjustments to enhance the acquisition, use, and allocation of resources to ensure growth and survival in adversity.

Sex differences present in plant leaves reflect sex-specific strategies due to differences in reproductive inputs, and may produce differences in sex tolerance under unfavorable conditions (Esp ́ito-Santo et al., 2003). Many studies have found differences in the growth strategies of male and female plants, albeit in different environmental conditions (Li et al., 2007; Juvany and Munn ́e-Bosch, 2015; Lei et al., 2017; Liu et al., 2020). In general, the proportional distribution of reproductive structures and aboveground nutrient growth in dioecious plants varies by sex and developmental stage (Teitel et al., 2016). This study confirmed that morphological variation in heteromorphic leaf is closely related to individual developmental stage and the increase in canopy height. At different developmental stages and canopy heights, LA, LT, and SLA were significantly higher in male plants than in female plants. Under drought conditions, males maintained higher LA and lower SLA than females (Xu et al., 2008a). As the height of tree increases, water stress is induced by increased gravity and path length resistance (Koch et al., 2004). It has been shown that large and thick leaves help to increase the distance and resistance of water diffusion from the interior of the leaf to the surface of leaf to reduce water loss and enhance carbon capture to adapt to the water stress caused by tree height (Zhai et al., 2020), while reducing SLA to enhance drought resistance (Ne'eman et al., 2011), and facilitate its survival. Thus, male plants are more tolerant to water stress caused by tree height, while female plants are relatively weaker. Considering the distribution of effective light at different canopy heights (Peavey et al., 2020), LA, LT, and LDW were much higher at the top canopy for both male and female plants than at the bottom canopy, indicating that increasing canopy height had a significant effect on leaf morphology.

Plant leaves can be morphologically adapted to different light conditions, and male plants are more susceptible to photo-inhibition than female plants (S ́anchez-Vilas and Retuerto, 2009). Wu et al. (2021) found that plants with high resource acquisition capacity can compensate for the inhibitory effects of environmental stress on their growth. In addition, photosynthetic properties of plant leaf area are related to tree height and degree of drought stress (Kenzo et al., 2015). This study showed that P_n , T_r , and g_s of heteromorphic leaves of both male and female plants were significantly and positively correlated with LA, LDW, LT, and CH, indicating that changes in stomatal exchange parameters were related to those in leaf morphology and canopy height. At different canopy heights, there were sex differences in P_n , T_r , and g_s with male plants at the same canopy height, which had higher values. Previous studies of *Pistacia lentiscus* L. found that males showed higher photosynthesis and stomatal conductance than females (Correia and D ́az, 2004), which is consistent with our findings. In addition, water stress caused by increased tree height may ultimately limit leaf expansion and photosynthesis (Koch et al., 2004; He et al., 2008; Zhang et al., 2009; Zhai et al. 2020). Therefore, male plants have a high photosynthetic capacity, which may facilitate the acquisition and use of carbon resources in adversity.

In some cases, plants exhibit more conservative strategies in terms of water use under unfavourable conditions (Retuerto et al., 2000; Alvarez-Cansino et al., 2010; Grossiord et al., 2017). Koch et al. (2004) suggest that water potential decreases with increasing tree height and that reduced LWP leads to reduced growth, assimilation, and hydraulic conductivity (Ratzmann et al., 2019). The increase in plant leaf $\delta^{13}C$ favors photosynthetic productivity in bright and dry upper canopy conditions (Kenzo et al., 2015). In this study, we found that $\delta^{13}C$ of heteromorphic leaves was significantly and positively correlated with CH, and LWP was negatively and significantly correlated with CH. As canopy height increased, LWP significantly decreased and $\delta^{13}C$ significantly increased in both male and female plants, which supports the above results. In addition, at the same developmental stage and canopy height, there were sex differences in heteromorphic leaf $\delta^{13}C$ and LWP, with female plants having higher heteromorphic leaf $\delta^{13}C$ and LWP than male plants. Under relatively well-watered conditions, the efficient transport of water

through the xylem allows for higher rates of carbon acquisition and thus conversion to more effective carbohydrates, while female leaves appear to have high metabolic costs that can be supported by larger water transport requirements (Esp fito-Santo, 2003), however, this acquisition strategy may have long-term costs. Females have a more sensitive water balance and more liberal water use efficiency than males, which may help compensate for female reproductive costs (Obeso, 2002; Barrett and Hough, 2013; Lei et al., 2017). Dawson and Bliss (1989) found that the potential water use efficiency of female *Salix* is higher in arid habitats. In addition, the heteromorphic leaf LA and SLA of female plants were all significantly and negatively correlated with LWP and $\delta^{13}\text{C}$, suggesting that sex differences in heteromorphic leaf $\delta^{13}\text{C}$ and LWP are related to leaf morphological adjustment. This shows that female plants can be constructed by altering leaf morphology to enhance the long-term efficiency of water use to meet greater growth benefits.

In higher plants, water stress causes the accumulation of osmoregulatory substances such as Pro and MDA in response to the stress caused by water scarcity (Kishor et al., 2005). In general, MDA is an indicator of the degree of lipid peroxidation induced by oxidative stress, and is important in plant adaptation to environmental stress (Apel and Hirt, 2004; Xu et al., 2008b; Sies and Jones, 2020). Previous studies have shown that drought stress significantly increased leaf MDA concentrations in both male and female plants (Xu et al., 2008b). This study showed that the concentrations of Pro and MDA in heteromorphic leaves were significantly and positively correlated with CH, LA, LDW, and LT. As the height of canopy increased, the concentrations of Pro and MDA in both male and female plants tended to increase, and were significantly higher at the top of the canopy than at the bottom of the canopy. This indicates that as canopy height increases, the degree of water deprivation increases, and thus more water stress is experienced, which has a significant effect on the morphological construction of heteromorphic leaves and the accumulation of osmoregulatory substances. In addition, there were gender differences in MDA concentrations of heteromorphic leaves at the same canopy height, with heteromorphic leaves having higher MDA concentrations in male than in females, indicating that male plants are more sensitive to water deprivation.

Early evidence suggests that male plants tend to be more abundant than females under unfavourable environmental conditions (Hutline et al., 2016; Melnikova et al., 2017). Sex differences may reflect trade-offs between resource-exploration strategies across resource gradients (Lei et al., 2017), in which female plants choose to improve long-term water use and meet greater reproductive costs through leaf morphological adjustments, while male plants choose to engage in efficient nutrient storage and enhance organic carbon acquisition and water uptake through synergistic changes in heteromorphic leaf morphology and physiology that improve stress tolerance and the chances of survival in under-resourced habitats (Hultine et al., 2016). Differences in resource uptake, allocation, and utilization strategies between male and female plants may be an evolved response to meet specific resource needs associated with reproduction, thereby maintaining the population structure and stability of ecosystem.

5 Conclusions

This study revealed significant differences in morphological and physiological characteristics of heteromorphic leaves of *P. euphratica* to accommodate different developmental stages and canopy heights. With increasing developmental stage and canopy height, on the one hand, male plants had a greater photosynthetic, water-absorbing capacity compared with female plants, thus increasing access to and utilization of organic carbon resources under adverse conditions, further improving stress tolerance and chances of survival under unfavorable habitats. On the other hand, female plants had relatively less adjusted leaf morphology but greater long-term water utilization as a means of meeting or compensating for greater growth benefits. In addition, differences in resource uptake, allocation, and utilization strategies between male and female plants were associated with individual developmental stages and increased canopy height. Therefore, male

and female *P. euphratica* evolved responses to meet specific reproduction-related resource requirements through feedback regulation between heteromorphic leaf morphological adjustments and physiological responses to adapt to changes in individual developmental stages and canopy height. The results of this study might provide a basis for the management of dioecious plant population.

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Appendix

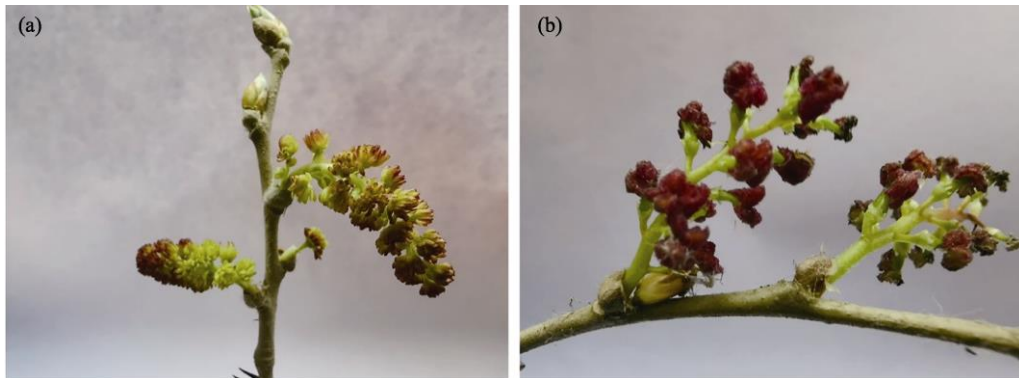


Fig. S1 Female and male inflorescences of *P. euphratica*. (a), male inflorescence; (b), female inflorescence.

Table S1 Basic information of male and female *P. euphratica*

Sex	Diameter class (cm)	Average diameter at breast height (cm)	Average tree height (m)	Average tree age (a)
Female	8	8.33 ^e	7.53 ^e	8.10 ^e
	12	14.30 ^d	9.47 ^d	9.30 ^d
	16	17.67 ^c	11.27 ^b	10.37 ^b
	20	23.23 ^{ab}	12.87 ^a	11.17 ^a
Male	8	9.33 ^e	7.97 ^e	8.37 ^e
	12	14.37 ^d	10.00 ^{cd}	9.70 ^{cd}
	16	17.33 ^c	10.93 ^{bc}	10.13 ^{bc}
	20	24.83 ^a	12.70 ^a	11.10 ^a

Note: Different lowercase letters within the same column indicate significant difference among females or males at $P < 0.05$ level.